

BIOLOGICAL FALSE POSITIVE WASSERMANN REACTIONS IN UGANDA*

BY

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In a country like Uganda where syphilis is common, yaws occurs, and many of the diseases known to cause false positive Wassermann reactions (such as malaria, leprosy, relapsing fever, smallpox vaccination, trypanosomiasis, lymphogranuloma venereum, and infectious hepatitis) exist, it seemed desirable to obtain some background information about the frequency of positive serological tests for syphilis in the general population in order that the results of tests on individual patients might be evaluated.

As the *Treponema pallidum* immobilization (TPI) test was not available, the validity of the Wassermann reaction (WR) was assessed against the Reiter protein complement-fixation (RPCF) test. Personal experience in Great Britain had shown that the majority of sera giving a positive WR but a negative RPCF test also have a negative TPI test, and a large series of tests has shown the RPCF test to be highly specific (Sequeira 1960). For the purpose of this study a positive WR unsupported by a positive RPCF test is regarded as a biological false positive (BFP) reaction. As was anticipated, BFP reactions occurred with a considerable proportion of sera tested and this paper is mainly concerned with an investigation of the cause of these reactions.

Material and Methods

Blood was taken by venepuncture with a disposable syringe and transferred to a plastic test tube, and the serum when separated was stored in a fresh plastic tube at -20°C . Samples were tested from the following groups of the population:

- (1) Hospital patients not suspected of having syphilis or yaws and blood donors in the Kampala area — these were of various social backgrounds such as prisoners, policemen, school children, and civil servants (Total 627).
- (2) Leprosy patients at the St. Francis Leprosarium, Buluba, about 70 miles to the east of Kampala (Total 475).

- (3) Hospital out-patients in Kabale (Total 259). Kabale is in south-west Uganda, at an altitude 6,000 feet, and is non-malarious. Kampala is moderately malarious.
- (4) Persons whose malaria antibody titre as tested by the method of Voller and Bray (1962) was known (Total 85).
- (5) Children with kwashiorkor (Total 139).
- (6) Makerere University College students.

The antigens used in testing all serum samples were:

- (1) Burroughs Wellcome Standard WR antigen.
- (2) Organon Reiter protein antigen diluted 1/40 with normal saline.

WR and RPCF tests were carried out using a dropping technique with 0.1 ml. unit volumes in perspex haemagglutination trays. A single 1 in 5 dilution of inactivated serum was incubated at 37°C . for 2 hours with antigen and $1\frac{1}{2}$ m.h.d. guinea-pig complement. Sheep cells sensitized with 5 m.h.d. anti-sheep cell serum were then added and the results read after 30 min. incubation at 37°C . A control consisting of serum without antigen was included on each occasion.

Results

It was immediately evident that the proportion of serum samples reacting in various ways varied with the age of the patients. The results are therefore given grouped by decades. The results of the WRs and RPCF tests on the normal populations of Kampala and Kabale are shown in Tables I and II.

TABLE I
627 NORMAL BLOOD DONORS AND HOSPITAL OUT-PATIENTS IN THE KAMPALA AREA

Decade of Age	Number of Persons Reacting				Total
	WR+ RP+	WR- RP+	WR+ RP-	WR- RP-	
1st	2 (2.6)	0	24 (31.5)	50 (66.0)	76
2nd	4 (2.7)	3 (2.0)	26 (17.0)	117 (78.0)	150
3rd	14 (6.9)	8 (4.0)	36 (17.8)	145 (71.0)	203
4th	7 (7.0)	12 (12.0)	13 (13.0)	67 (68.0)	99
5th	8 (16.0)	4 (8.0)	6 (12.0)	32 (64.0)	50
6th	7 (14.0)	3 (6.0)	3 (6.0)	36 (72.0)	49
All Ages	42 (7.0)	30 (5.0)	108 (17.0)	447 (71.0)	627

Percentage of total in brackets.

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TABLE II
259 HOSPITAL OUT-PATIENTS AT KABALE

Decade of Age	Number of Persons Reacting				Total
	WR+ RP+	WR- RP+	WR+ RP-	WR- RP-	
1st	0	0	14 (56.0)	11 (44.0)	25
2nd	9 (7.0)	2 (1.5)	55 (44.0)	59 (47.0)	125
3rd	3 (5.0)	0	10 (17.0)	46 (78.0)	59
4th	1 (3.5)	2 (7.0)	3 (10.0)	23 (80.0)	29
5th	0	1 (7.0)	2 (15.0)	10 (77.0)	13
6th	1 (12.0)	0	1 (12.0)	6 (76.0)	8
All Ages	14 (5.0)	5 (2.0)	85 (33.0)	155 (60.0)	259

Percentages of total in brackets.

Table III shows the results of tests on 475 patients with leprosy, of both lepromatous and tuberculoid types and of variable duration. Table IV shows the number of lepromatous and tuberculoid cases respectively giving BFP reactions.

TABLE III
475 CASES OF LEPROSY

Decade of Age	Number of Persons Reacting				Total
	WR+ RP+	WR- RP+	WR+ RP-	WR- RP-	
1st	5 (6.4)	0	38 (49.0)	35 (45.0)	78
2nd	7 (5.8)	0	41 (34.0)	72 (60.0)	120
3rd	9 (13.0)	6 (9.0)	12 (18.0)	40 (60.0)	67
4th	11 (9.3)	17 (14.4)	7 (5.9)	83 (70.0)	118
5th	4 (7.0)	1 (1.8)	4 (7.0)	48 (84.0)	57
6th	1 (2.0)	3 (8.6)	1 (2.9)	30 (85.5)	35
All Ages	37 (8.0)	27 (6.0)	103 (22.0)	308 (65.0)	475

Percentage of total in brackets.

TABLE IV
PROPORTION OF BFP REACTORS IN 473 PATIENTS WITH LEPROMATOUS AND TUBERCULOID LEPROSY

Type of Leprosy	Average Age (yrs)	No. of Patients	No. of BFPs
Lepromatous	33	87	7 (8)
Tuberculoid	26	386	92 (23)

Percentage of total in brackets.

Table V shows the number of BFP reactions obtained using serum samples from the group of persons with known malaria antibody titres, and Table VI the number of BFP reactions obtained with 86 samples of serum from patients at Kabale with a known vaccination history.

Discussion

(1) The incidence of serological evidence of treponemal infection in the normal population as judged by a positive RPCF test is considerable, a point which must be borne in mind when interpreting the result of a test in an individual patient. The overall incidence amongst 627 persons in the Kampala area was 12 per cent. The incidence in children under 10

TABLE V
BFP REACTIONS WITH SERUM OF PERSONS WITH KNOWN MALARIA ANTIBODY TITRE

Malaria Antibody Titre	No. Tested	BFPs
Less than 1/100	35	7 (2)
1/100 to 1/800	24	11 (46)
Greater than 1/800	26	19 (73)

Percentage of total in brackets.

TABLE VI
BFP REACTIONS WITH SERUM SAMPLES OF PERSONS AT KABALE WITH A KNOWN VACCINATION HISTORY

Vaccination History	No. tested	BFPs
Not vaccinated during previous 3 months	25	3 (12)
Vaccinated with recent scar visible	26	3 (12)
Vaccinated but with no recent scar	35	11 (31)

Percentage of total in brackets.

years of age, which presumably represents evidence of congenital syphilis or yaws, was 2.6 per cent., and the steady post-pubertal rise to a level of 20 per cent. in the fifth decade is presumably the result of venereal syphilis. The incidence of serological evidence of treponemal infection amongst leprosy patients is very similar to that of the Kampala population but two points may be noted: the higher incidence in children under 10 years and the lower incidence in adults over 40 years of age. The former suggests that although yaws is now considered a rare clinical condition, it is responsible for a proportion of the serologically positive children, since it might be expected that children living in social conditions in which they contract leprosy would also be more liable to a contact infection like yaws. The latter observation may perhaps be explained by the fact that the leprosy patients were under constant medical supervision and received large doses of penicillin as part of the treatment of septic trophic lesions which incidentally cured their syphilis in a proportion of them.

(2) The overall incidence of 17 per cent. BFP reactors in the normal Kampala population renders the WR a test of little use in the diagnosis of syphilis. It is interesting that the BFP rate is greatest in the first decade and declines steadily with advancing age. All the populations in which reasonably large numbers of persons were tested exhibit this phenomenon, the cause or causes of which must now be considered.

(a) Leprosy is generally supposed to cause BFP reactions in a considerable proportion of patients. The actual figures given by different workers have varied considerably as would be expected from the varying

sensitivity of different tests for reagin. For example, Edmundson, Wolcott, Olansky, and Ross (1954), in a study of 224 patients, found 63·4 per cent. positive with the Kolmer type WR when the *Treponema pallidum* immobilization test was positive in only 11·2 per cent. Many other workers have confirmed the frequency of BFP reactions in leprosy (Cannefax, Ross, and Bancroft, 1959; Nelson, 1952; Daguet and Languillon, 1961). Our findings confirm the high frequency of BFP reactions in leprosy cases, the overall incidence being 22 per cent. But it is doubtful whether BFP reactions are more frequent amongst them than in the general population of Uganda, their incidence falling between that of two normal populations, Kampala (17 per cent.) and Kabale (33 per cent.).

Most reports indicate that BFP reactors are more common in lepromatous than in tuberculoid leprosy (Edmundson and others, 1954; Daguet and Languillon, 1961), but this does not seem to be true in Uganda, where the BFP rate for lepromatous leprosy was 8 per cent. and for tuberculoid leprosy 23 per cent. However, as has been shown, age is a most important factor affecting the BFP reactor rate, and many of our cases of tuberculoid leprosy were children, the average age being 26 years as opposed to 33 years for the lepromatous cases, and this may account for the higher incidence of BFP reactors rather than the type of the disease.

(b) Malaria is stated to cause BFP reactions, possibly in 100 per cent. of cases (Sequeira, 1960). This disease is very common in Uganda although the incidence of infection varies very much in different parts of the country. Malaria might therefore, be expected to be an important contributory cause to our high BFP reactor rate. In our attempt to assess this we tested 84 samples of serum with a known malaria antibody titre and have found an increasing proportion of BFP reactors with increasing titres of malaria antibody. Malaria may therefore cause some of our BFP reactions. However, the very high BFP reactor rate in the population of the non-malarious Kabale area, a sample of whom were shown to have no malaria antibody, suggests that it is not a major cause.

(c) Vaccination against smallpox is said to cause a BFP reaction in 20 per cent. of cases (Sequeira, 1960). During the period of this investigation there was an outbreak of smallpox in Uganda and we did not appreciate until late in our investigation that widespread mass vaccination campaigns were being carried out. We are, however, able to offer a little evidence as to the importance of vaccination as a cause of BFP reactions. The vaccination campaign in the Kabale area had been particularly thorough, and questioning of a random sample of 200 hospital out-patients revealed that 80 per cent. had been vaccinated within the last 3 months. In 86 cases from whom we took a blood sample we also inquired whether or not they had been vaccinated within the past 3 months and we examined those who answered in the affirmative for evidence of a recent vaccination scar. The numbers involved were small but there is no striking difference in the BFP reactor rate between the vaccinated with a recent scar and the unvaccinated. The average age

of the people involved was very similar. Moreover vaccination was carried out fairly uniformly on the population and, in particular, all our leprosy patients had been recently vaccinated, and yet there is still the strikingly different incidence of BFP reactions with age.

Our evidence with respect to leprosy, malaria, and vaccination as causes of BFP reactions is equivocal. Our most interesting finding is that the BFP reactor rate varies with age, being highest in children. It seems likely to us that there may be many causes of BFP reactions operative in Uganda and that perhaps the high rate in children is a reflection of the activity of their antibody-producing mechanisms responding to the repeated assault of a great variety of infectious agents to which they subsequently acquire immunity. There may be a single important cause accounting for most of the BFP reactions, but if so we are unable to suggest what it might be. One possible cause which did occur to us was malnutrition, particularly protein malnutrition which produces changes in the plasma proteins.

A study of the ages of the child BFP reactors in more detail does not afford support for the idea that protein malnutrition is an important factor, since we have found BFP reactions in several children under 1 year of age and the highest incidence of BFP reactions occurs at an older age than the highest incidence of malnutrition. Nonetheless we tested serum from 139 cases of untreated kwashiorkor and found 40 per cent. BFP reactors, a figure not different from the normal population of corresponding age. Moreover, the incidence of BFP reactors amongst a small number of Makerere students who were receiving a balanced diet in halls of residence seems to be the same as that amongst the general population in their age group.

Summary

Because of the expected frequency of positive serological tests for syphilis in the population of Uganda due both to treponemal infection and to biologically false positive (BFP) reactions, a total of 1,650 blood samples from persons not suspected of treponemal infection and drawn from various groups of the population have been tested by the Wassermann reaction and Reiter protein complement-fixation test in order to provide background information for the interpretation of tests in individual patients. About 15 per cent. of the adult population tested in this study show serological evidence of treponemal infections, probably syphilis. BFP reactions are also very common, but are most frequent in children and decrease in frequency with age. The relation of these BFP reactions to leprosy, malaria, smallpox vaccination, and malnutrition is

discussed, and it is concluded that these conditions probably account for only a minority of BFP reactions.

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Les réactions biologiques pseudo-positives du test Wassermann en Ouganda

RÉSUMÉ

Vu la fréquence connue des tests sériques positifs de la syphilis chez les habitants de l'Ouganda, dûs à l'infection par le tréponème et aussi aux réactions biologiques pseudo-positives (BPP), un total de 1650 échantillons de sang obtenus de personnes chez qui on ne soupçonnait pas la présence d'une infection par le tréponème et choisis de différents groupes de la population, a subi le test Wassermann et le test de fixation du complément de la protéine de Reiter afin de fournir des renseignements de base pour l'interprétation des tests chez chaque malade. A peu près 15 pour cent de la population adulte soumis à ce test dans cette étude a montré des signes sériques d'infections par le tréponème, probablement celui de la syphilis. Les réactions BPP sont aussi très communes, mais beaucoup plus fréquentes chez les enfants et diminuent avec l'âge. La relation des réactions BPP à la lèpre, la malaria, la vaccination anti-variolique et la malnutrition est discutée, et il a été conclu que ces conditions expliquent probablement qu'une minorité des réactions biologiques pseudo-positives.